Journal of Chemistry Science
ISSN: 3078-736X
DOI: https://doi.org/10.61784/jcs3002

BODY THERMOREGULATORY ADAPTATION AND BLOOD SERUM MINERAL METABOLIC PROFILE OF RABBITS SUPPLEMENTED CAMEL'S FOOT (SCHUM) SEED ESSENTIAL OIL BASED DIET

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Abstract: The purpose of the current investigation was to ascertain the impact of the essential oil derived from Schum (*Piliostigma thonningii*) seeds on body thermoregulation and serum mineral parameters in the bucks. Three treatment groups of fifteen rabbits each were randomly assigned to the treatments and balanced for their body weight (BW) so that the average beginning weight of the rabbits in each group was similar. The average initial body weight was 0.27 kg in a fully randomised experimental design. Treatment 1 was a basal control diet. In treatments 2 and 3, the basal control diet was supplemented with 2 ml PEO/kg diet and 4 ml PEO/kg diet respectively. Serum bicarbonate increased ($P < 0.05$) with increasing level of PEO supplementation. While serum calcium, potassium, sodium, chlorine was higher ($P < 0.05$) in T2 and T3 than in T1. Serum phosphorus was higher ($P < 0.05$) in T3 than T1 with T2 being intermediate ($P > 0.05$) between T1 and T3. Rectal temperature was greater ($P < 0.05$) in T1 than in T2 and T3. Serum magnesium, heart and respiratory rates were not affected ($P > 0.05$) by treatments. It was therefore concluded that *P. thonningii* essential oil supplementation enhanced body thermoregulation by decreasing the experimental animal's temperature and serum minerals profile of the experimental rabbits. However, 4 ml PEO/kg diet is the optimum supplementation level, as it was more effective in enhancing the physiological health.
Keywords: Bucks; Tropical phytogenic feed supplement; Body vitals; Serum minerals

1 INTRODUCTION

Rabbits are averagely small sized livestock that are quite fairy easy and cheap to manage at minimal cost. They can be raised and adequately managed on backyard farm or on large scale production. They produce white palatable meat, moderately higher protein (about 21%), lower fat, lower cholesterol and well flavored similar to poultry [1].
High temperature (heat) is a critically transient climatic factor affecting livestock especially rabbit production in the tropics. The rabbit is very largely subservient on respiratory evaporation for the physiological control of its general body temperature and this imparts only a limited power of adaption to hot climates [2, 3]. Heat is also released by radiation and convection, but these are considerably restricted by the rabbit's furry covering. [4], reported that short hair and larger ears physically and physiologically functions to assist the cooling process in New Zealand White rabbits. According to these workers, growth and development were impaired by atmospheric (surrounding) temperatures of 28.3°C and above. Generally, the higher the surrounding (ambient) temperature, the greater the agitative disruption of the rabbit's physiological activities and functions.
There is adequate documentation and corroboration that high ambient temperatures can mar the general performance of rabbits. [1], stated the presence of the secondary metabolites in *Piliostigma thonningii* plant and its extracts was of industrial and medicinal importance suggests its potency in body physiology, linking to the recent research findings revealing its normal flavouring, antioxidant, insecticidal, and antibacterial qualities [2].
The jeopardy of abuse of synthetic antibiotics and lack of adherence to withdrawal periods in Africa, especially in Nigeria, occasioned with the current epidermic of antimicrobial resistance challenge, as well as the subsequent ban on antibiotic growth promoters by several countries have compelled the search for alternatives (plant-based antioxidants and antimicrobials), for improving animal productivity and minimizing adverse effects on human consumers. Due to this ban, a great deal of study has been done to explore the use of phytogenics as alternate feed additives in animal nutrition and physiology [3].
There is no information on the body thermoregulatory and serum mineral parameters of rabbits supplemented PEO. The objectives of the present research were, therefore, to: 1) Evaluate the thermoregulation parameters and 2) Determine the serum mineral profile of rabbits fed PEO supplemented diet.

2 MATERIALS AND METHODS

2.1 Assemblage of *Piliostigma thonningii* Seeds and Subsequent Essential Oil Extraction

Piliostigma thonningii seeds were carefully obtained from within the Northern parts of Nigeria and surroundings and they were later verified by a licensed taxonomist at the Department of Biological Science at the Forestry Research Institute of Nigeria.

The Clevenger apparatus was used to extract the essential oil in accordance with [2] technique. The *P. thonningii* seeds were carefully ground, dried in enough shade, and kept at room temperature until they were needed again (extraction process). Laboratory procedure involved placing the ground seed sample in a steel apparatus and allowing it to soften and produce the essential oil in vaporized form by intermittently heating up after connecting the condenser to a water inlet and outlet, precisely 100 g of dried ground sample was suspended in precisely 700 ml of distilled water using distillation process at about 100°C for three hours. The resultant vaporized essential oil droplets were congregated in a cooling system after prior mixing with steam and passage through the carrier.

2.2 Experimental Site

The study was conducted at the University of Abuja Teaching and Research Farm's Monogastric Unit, which is situated in Giri inside the Gwagwalada Area Council in the Federal Capital Territory of Abuja, Nigeria. The project site lies between latitudes $08^0$51' and $09^0$37'N and longitudes $007^0$20' and $007^0$51' E. Annual rainfall ranges from 1,145 – 1,631 mm. The temperature in dry season is between 36 – 42℃ and 25.8 – 30.2℃ during the raining season. Relative humidity is about 60% during the raining season and 30% during dry season [5].

2.3 Experimental Animals, Management and Treatment

For the experiment, 45 clinically certified healthy weaned male Dutch rabbits weighing an average of 0.27 kg were used, they were about five weeks old. A reliable source (The National Animal Production Research Institute at Ahmadu Bello University in Zaria, Nigeria), is where the rabbits were bought. Two weeks prior to the arrival of the rabbits, the hutches and Hypo® (sodium hypochlorite, caustic soda, and de-mineralized water) and antiseptic (Morigad) were used to sterilize and disinfect the area around them. The animals received preventative medication and were placed in quarantine for exactly two weeks. The prophylactic measures comprised injecting a subcutaneous dose of an anti-parasitic medication (Avomec®) at 0.5 mg/kg of the animal body weight (BW) to control endo and ecto parasites, administering an anti-stress drink (Vitalyte®), and administering a parenterally administered intramuscular injection of the broad-spectrum antibiotic oxytetracycline HCl at 1.0 mL/10 kg BW. Additionally, at the beginning of the trial, rabbits received a single subcutaneous treatment with coccidiostat (Sulphadimidine Sodium BP solution) at a dose of 1 mL/rabbit per manufacturer's advice.

Daily sanitation of the intermittent hutches was carried out with a strong disinfectant. With precisely fifteen rabbits in each group, the rabbits were systematically divided into three groups. After balancing for body weight (BW), the rabbits in each group were assigned to one of three treatments in a completely randomised manner, with their beginning BWs being numerically equal.

Based on the guidelines provided by the [6], a base control diet was formulated for growing rabbits. For a duration of 12 weeks, water and feed were given freely, with feeding occurring twice a day at 8:00 and 16:00. In the initial treatment, a baseline control food was given to the rabbits. In the other treatments, two and four millilitres of PEO per kilogram of the control food were added.

2.4 Body Thermoregulation

Rectal temperature, respiratory rate and heart rate of individual rabbit were measured twice a week at 11:00 hrs. Rectal temperature was measured using digital thermometer. The sensory tip was disinfected with an antiseptic, lubed with petroleum jelly (Vaseline) and then inserted into the rectum of individual animal at a depth of 2 cm and at the display of constant temperature indicated by “C L0” on the thermometers’ mini digital screen. After the beeping sound of the alarm signal of the digital thermometer, the thermometer was removed from the rectum and the displayed body temperature was recorded [7]. Respiratory rate was determined by counting the number of abdominal movements per minute using the seconds hand in an analogue wrist watch for one minute and the counts recorded. Heart rate was determined using a stethoscope placed at the left side of the ribs (the anatomical location of the heart). The normal sound of the heart was the “lob dob” sound which indicated a complete heart beat for a minute using the seconds hand in an analogue wrist watch for one minute and the rates recorded appropriately as described by [7,8].

2.5 Blood Collection and Analysis

Blood samples were taken from each treatment's individual rabbits on the last day of the trial. Before the rabbits in each treatment group had access to food and water in the morning, blood samples were drawn from the marginal veins in their ears. After being collected into multiple 5 ml vacuum tubes and chilled using ice packs, the blood samples were promptly transported to the laboratory for examination. Four hours after collection, Serum calcium, sodium, potassium, phosphorus, chlorine, magnesium, bicarbonate was analyzed by calorimetric methods using Bio Maxima reagent sets (Lublin, Poland) in a Metrolab 5.0, Norway, Oslo.

2.6 Statistical Analyses

Data on haematological parameters and semen characteristics were subjected to analysis of variance in a completely randomized design using the SPSS (23.0). The same software's Duncan multiple range test was performed to assess the significance of the mean difference at the $P \leq 0.05$ level.
The statistical model is shown below

$$Yij = \mu + tij + eij \quad (1)$$

Where:
Yij = the overall response to the specific parameter under investigation,
μ = the general mean peculiar to each observation,
tij = the fixed effect of the dietary treatments (i = 3) on the observed parameters and
eij = the random error term for each estimate

3 RESULTS

3.1 Body Thermoregulation of Rabbits Fed *Piliostigma thonningii* Essential Oil Supplemented Diet

Table 1 shows body thermoregulation of rabbits fed PEO supplemented diet. Rectal temperature varied from 38.15 to 40.00℃ and higher ($P < 0.05$) in T1 compared to T2 and T3 which were unaffected ($P > 0.05$). Heart rate varied from 200.11 to 206.63 beats per minutes and respiratory rate from 2.34 to 55.67 cycles per minute. Both parameters were unaffected ($P > 0.05$) by the treatments.

Table 1 Body Thermoregulation of Rabbits Fed *Piliostigma thonningii* Essential Oil Supplemented Diet

Parameters	Treatments				
	T1	T2	T3	SEM	RV
Rectal temperature (^{0}C)	40.00^a	38.23^b	38.15^b	0.77	37.5-40.0
Heart rate (bpm)	206.63	202.31	200.11	1.81	130-325
Respiratory rate (cpm)	55.67	52.34	53.01	0.75	30-60

Note: Means with the different superscripts along the row are significantly (P < 0.05) different T1, T2 and T3 connoting treatments one, two and three. T1, 0 ml P. thonningii essential oil; T2, 2 ml *P. thonningii* essential oil/kg diet; T3, 4 ml P. thonningii essential oil. RV: Reference values as stated by Hassan and Hassan (2003)

3.2 Serum Mineral of Rabbits Fed Piliostigma Thonningii Essential Oil Supplemented Diet

Table 2 shows the serum minerals of rabbits fed PEO supplemented diet. Serum calcium (1.78 – 2.62 Mmol/L), potassium (4.00 – 5.16 Mmol/L), sodium (132.58 – 152.90 Mmol/L) and chlorine (92.65 – 105.68 Mmol/L) were lower in T1 ($P < 0.05$) than T2 and T3 which were similar ($P > 0.05$). Serum phosphorus varied from 1.13 to 1.84 Mmol/L in T1 to T3, with a higher ($P < 0.05$) value in T3 than T1 but similar ($P > 0.05$) values for T1 and T2, and T2 and T3. Serum bicarbonate varied from 16.92 – 36.20 Mmol/L, and was highest in T3 and lowest in T1 ($P < 0.05$). Serum magnesium (0.94 – 1.16 Mmol/L) was not ($P > 0.05$) affected by the treatments.

Table 2 Serum Mineral Parameters of Rabbits Fed Piliostigma Thonningii Essential Oil Supplemented Diet

Parameter (Mmol/L)	T1	T2	T3	SEM	RV
Calcuim	1.78^b	2.61^a	2.62^a	0.27	1.4-3.1
Potassium	4.00^b	4.74^a	5.16^a	0.26	3.6-6.9
Phosphorus	1.13^b	1.52^{ab}	1.84^a	0.26	1.3-2.2
Sodium	132.58^b	145.72^a	152.90^a	3.94	131-155
Chlorine	92.65^b	101.35^a	105.68^a	2.23	92-112
Bicarbonate	16.92^c	25.57^b	36.20^a	2.73	16-38
Magnesuim	0.94	1.04	1.16	0.09	0.8-1.2

Means with the different superscripts along the row are significantly (P < 0.05) different T1, 0 ml administration of P. thonningii essential oil; T2, 2 ml of P. thonningii essential oil; T3, 4 ml of P. thonningii essential oil/kg diet. RV; reference values as stated Beers, M.H. 2006. The Merck manual of diagnosis and therapy. 18th ed. Whitehouse station, N.J.: Merck Research laboratories.

4 DISCUSSIONS

4.1 Body Thermoregulation of Rabbits Fed Piliostigma thonningii Essential Oil Supplemented Diet

Heat stress is an important stressor that affects productive performance, and the use of ecofriendly dietary additives to alleviate the negative impacts of heat stress remains a vital issue [9]. Respiratory rate (RR), heart rate (HR) and rectal temperature (RT) are parameters that describe the physiological adaptation mechanism. According to [10], haematological parameters are related to the RT and RR in domestic animals. The RT, HR and RR of the experimental animals were within normal ranges for healthy rabbits [9]. This could be hinged on the fact that the experimental rabbits were apparently healthy and did not show any major sign of illness during the experiment. The normal ranges for healthy rabbits as stipulated by [11] and Veterinary Science from UC Davis for RT, HR and RR are 37.5 – 39.5 degrees Celsius, 130 – 325 beats per minute (bpm) and 32 – 60 cycles per minute (cpm), respectively. The results were also within stipulated references of 38.6 – 40.1 °C, average of 210 - 230 bpm and average 45 cpm by [11] for the RT, HR and RR respectively.

The lower rectal temperature in rabbits supplemented with 2 ml and 4 ml PEO proves the essential oil's antioxidative ability to reduce reactive oxygen species and their subsequent reaction with the body cells. Rectal temperature is regarded as a good index of the body temperature, even though there is considerable variation in different parts of the core of the body at different times [12]. According to [13], the normal temperature range of rabbit were reported to be between 38.0° C and 40.5° C. Generally, heat balance of the body can be affected by the body internal and external conditions. Internal conditions include the physiological processes in the body such as metabolic processes of feed. In this study, the addition of PEO in the feed had a significant impact on the rabbits' RT, although it was still within the stipulated range. [14] also reported that an increase in RT of an animal indicates the level of heat stress upon the animal. Therefore, elevation in the RT for rabbits indicates that they may be heat stressed. In general, the results of the RT were within range and in agreement with previous findings of [15] who reported that the dietary inclusion of extra virgin olive oil (EVOO), betaine (BET), lemongrass essential oil (LGEO), gallic acid (GA), vitamin C (VC) and vitamin E (VE) feed additives, especially EVOO, had a lowering effect on various rabbit temperature.

Respiratory rate of the experimental animals showed no significant difference among treatments and was within normal ranges for healthy rabbits. [16] stated that RR can be used to estimate the adverse effects of environmental temperature and is also an indicator of heat stress. [12] also reported that RR is a practical and reliable measure of heat load, and RR above normal value or range is an indicator of heat stress. Therefore, the normalcy in the respiratory rates of the rabbits irrespective of the diets is an indication that the animals were apparently healthy and not under heat stress. Heart rate of the experimental animals which was within stipulated range for healthy rabbits, suggests efficient recirculation of blood to peripheral tissues during the entire experiment.

4.2 Serum Mineral of Rabbits Fed Piliostigma Thonningii Essential Oil Supplemented Diet

The values for serum calcium, phosphorus, sodium, chlorine, bicarbonate and magnesium were within the normal reference range for clinically healthy rabbits as stipulated by [10, 17].

Calcuim imbalance (low level calcium) in domestic animals causes hyperparathyroidism, hypervitaminosis D, multiple myoloma and neoplastic disease or osteomalacia, rickets and renal failure [8]. The significantly higher (T2 and T3) and maintenance of the normal serum Ca levels within stipulated reference ranges in experimental animals imply that the treatments from this study did not have any deleterious effects or cause renal failure [18].

Serum phosphorus level was higher in T3 supplemented with 4 ml PEO/kg diet but similar between T2 and T3. According to [19, 20], low level phosphorus could be implicated in bone dysfunction, leading to serum calcium unavailability and several disorders leading to coma and death.

Serum potassium was higher in treatment groups supplemented with PEO implying better nervous and muscular activity of rabbits compared to those in the control. Potassium and sodium must be maintained in a certain proportion in the blood. Hypo and hyperkalemia are the terminologies used to describe low and high potassium levels respectively. However, the occurrence of the serum Potassium levels were all within the stipulated range for healthy rabbits in all treatments ruled out the possibility of hypo and hyperkalemia.

[18] explained that maintenance of serum Na levels, as obtained in this study, suggests that experimental animals were able to maintain cellular tonicity fluid balance and pH, regulate metabolic processes as well as involved in regulation of neural and muscular function.

Furthermore, Na and K, as electrolytes, are used to assess renal functions and since they were within the normal ranges, it implies that normal body functions of the experimental rabbits were maintained. This indicates *P. thonningii* EO did not compromise renal functions.

Serum chlorine was higher in T2 and T3 but was however within the stipulated range for healthy animals, the result implies its function in the maintenance of acid-base balance and muscular control in combination with other serum minerals (such as sodium and potassium) in all experimental treatments.

Values obtained for bicarbonate was highest rabbits supplemented with 4 ml PEO. [21, 22] showed a positive relationship between low serum bicarbonate and chronic kidney disease; therefore, the normal serum bicarbonate within reference range for healthy rabbits indicates the absence of incidence of chronic kidney disease.

5 CONCLUSION

P. thonningii essential oil supplementation reduced rectal temperature but enhanced the blood serum profile of the rabbits.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

ETHICAL APPROVAL

Camel's foot (Piliostigma thonningii) seeds were sourced from within the Guinea Savannah agro ecological zone. The ethical guidelines for plant materials are followed in the study for experimentation. The Animal ethical guidelines are followed in the study for analysis.

AUTHORS' CONTRIBUTIONS

EUA: general write up, statistical analysis and discussion. JOA: Laboratory analysis and review

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